REMARKS/ARGUMENTS

Reconsideration of this application is requested. Claims 1-17 have been cancelled and new claims 18-47 are added. The following remarks correspond to the identified parts of the outstanding Action.

I. PRIORITY

A certified copy of the priority document GB 0402129.1 filed January 30, 2004 will be submitted shortly.

II. SPECIFICATION

The specification has been amended to correct the numbers of the recited U.S. patents. The proposed new title is "Therapeutically effective Peptides related to Preproinsulin".

III. CLAIM OBJECTIONS

The informalities objected to have been corrected in the new claims filed herewith.

IV. THE 35 USC 112 REJECTIONS

Claim 6 stand rejected under 35 USC 112, first paragraph, on alleged lack of enablement grounds and lack of written description. In response, and without conceding to the merit of this rejection, claim 6 has been cancelled and is not reproduced in the amended claims.

Claims 1, 3-5, 6, 9, 12, 13, 15 and 17 stand rejected under 35 USC 112, second paragraph, as allegedly indefinite, based on the use of the terms "comprising or consisting" and "having", in relation to the respective amino-acid sequences. In response, these terms have been avoided in the new claims. Reference to these changes in claim wording is provided in more detail hereinafter.

With reference to original claims 9 and 15 (corresponding to new claims 24 and 33), the use of tolerising agents is well known to those skilled in the art of immunology and is addressed in the present application in connection with the teaching concerning administration of the novel peptides (see page 20, lines 21-30).

The term "about" in original claims 12 and 13 is no longer present in the new claims presented herewith.

The objection to original claim 17 is avoided in its counterpart new claim 33 by addition of explanatory wording. Bearing in mind the fact that the ELISPOT methodology is found in the literature, as the Examiner points out elsewhere, the added wording would unquestionably have been presumed as implied in the methodology defined in original claim 17.

V. THE 35 USC 101 REJECTIONS

Claims 1-7 stand rejected under 35 USC 101 as allegedly directed to non-statutory subject matter. In view of the claims presented in the application as filed, the objections raised in the Office Action under reply were understandably directed primarily to the claims to peptides as distinct from those directed to the method of treatment. These objections are responded to below in relation to the peptide claims as now

presented in the new claims. However, as a preliminary to stating Applicant's standpoint on the peptide claims, it is important to stress a major difference of objective as between the subject application and the cited art. This is made clear in new method claim 18, which emphasizes the kind of therapy to which the present application is directed.

The claimed method has the object of arresting the development of T1DM in patients showing symptoms of this disease or of preventing such development in patients at risk of becoming diabetic at some future time. This protective treatment is very different from conventional insulin therapy in the various forms in which such therapy has been hitherto proposed. The difference lies in addressing the fundamental cause of the disease condition itself, by seeking to control the development of the autoimmune response which causes T1DM. This distinction is discussed hereinafter in reference to the art cited by the Examiner.

In the new claims filed herewith, the peptides are now defined in a way which avoids any 'product of nature' objection. The Examiner's helpful suggestion for amendment of the previous peptide claims is appreciated but, on reflection, is not considered to be the best solution of the definitional problem. It is true that the peptides of SEQ ID Nos 4,5, 6, 9 and 10 are present as part of the whole preproinsulin molecule (as shown in the attached Figure of the structure and complete amino-acid sequence thereof). But use of "consisting essentially of " terminology in the new claims distinguishes the claimed products from their prior existence as components of the naturally occurring intermediate polypeptide preproinsulin. For practical use in therapy, these peptides will of course be prepared as synthetic peptides rather than as materials

isolated from Nature. In this respect, the words of the amended claims have a narrower construction, in accordance with well-established US patent practice, and do not therefore include large extensions of the defined sequences such as are found in Nature or in relatively large fragments of the preproinsulin molecule. Withdrawal of this rejection is respectfully requested.

VI. THE ANTICIPATION REJECTIONS

Claims 1, 8, 11-14 stand rejected under 35 USC 102(b) as allegedly anticipated by Chance et al. Claims 1, 6, 3-4 stand rejected under 35 USC 102(b) as allegedly anticipated by Filvaroff et al. Those rejections are respectfully traversed.

In addition to the Chance et al and Filvaroff et al disclosures cited by the Examiner, Applicants now draw the Examiner's attention to the publication of Congia et al (Proc. Natl. Acad. Sci. U.S.A. vol 95 :3833-3838), a copy of which is supplied herewith by way of an IDS with IDS fee and PTO 1449, which describes an investigation of immunodominant T cell epitopes relevant to Type 1 diabetes. The cited art and Congia et al. will now be addressed.

Chance et al

This citation is of little relevance to the subject application. Chance et al are concerned with the design of polypeptides which may be used as alternatives to proinsulin insofar as they possess insulin-like activity. Firstly, the type of therapy to which this citation is directed is on a par with the conventional treatment of diabetic patients by administration of exogenous insulin in order to compensate for the reduced natural levels of this hormone in such patients. This is a totally different type of therapy

from that of the subject application, which is applied to counteract the autoimmune response which destroys the insulin-producing cells of the body.

Secondly, although one of the bridging peptides used by Chance et al to combine the A and B chains of insulin in their intended final product has a sequence similar to that of the core sequence described in the present application, this bridging sequence is not intended to be prepared or used otherwise than in covalent connection to the insulin A and B chains. The bridging peptide is not synthesised as a separate compound either in the synthetic method or in the gene-therapy method described in Chance et al. For the above reasons, the passages in Chance et al to which the Examiner has drawn attention have no bearing on the claims of the present application.

Filvaroff et al

This citation is also of no relevance to the claims of the present application as now claimed. Apart from the fact that Filvaroff et al is concerned only with cartilagenous disorders, the peptide sequences included in the SEQ ID Nos referred to by the Examiner are parts of much larger sequences which have no application to T1DM therapy of any kind, and especially the specific therapy to which the present invention is directed. Again, as in the above remarks on Chance et al, the modified sequence definition in the amended claims clearly distinguishes the present invention from the cited art.

Congia et al

The Congia et al publication reports on epitopes in preproinsulin which are claimed to be immunodominant epitopes. In the context used by these authors, immunodominance is an expression of the quantity of an epitope presented by an

antigen presenting cell. The immunodominant epitope would be that most represented from a given antigen. It is clear, therefore, that the term 'immunodominance' as used by Congia et al. does not indicate the potential for an epitope to be disease related, or to have potential therapeutic activity.

The Congia Abstract asserts LALEGSLQK as the immunodominant epitope relevant to T1DM. This sequence falls short of those of all the peptides claimed in the present application, of which SEQ ID No 9 (QPLALEGSLQK) is the central core sequence common to all the exemplified peptides SEQ ID Nos. 4, 5, 6, 9 and 10. It is evident from the investigations of the present inventor, described fully in the subject application, that the extension of the core sequence by addition of certain selected neighboring amino acids in the preproinsulin molecule preserves or enhances therapeutic activity in the resulting molecule.

In Congia et al., no data are presented on the reactivity of the peptide LALEGSLQK sequence which is claimed to be the most prevalent epitope from this particular antigen. Therefore, the claim to have identified this as an immunodominant epitope is unsupported. It will be known by those knowledgeable in the field of immunology that a peptide of 9 amino acids length (as LALEGSLQK is) is too short to bind to class II MHC molecules with any efficiency. It is improbable that a peptide of this length could be the target of effector T cells during disease development, or be induced to be the target of regulatory T cells during a therapeutic intervention. Indeed, the nearest peptide to this sequence that was tested in the study of Congia et al is LALEGSLQKRGI, which gives minimal/no reactivity in Fig 3.

Congia et al disclose no relationship between their recited peptides and human immunology/disease. No data on man or on patients with diabetes is presented. The paper would therefore not suggest to the skilled person that any of the peptides they have identified might have therapeutic potential. Despite what they say in the penultimate sentence of their Abstract, no studies on man are presented and no link to human diabetes is presented. Without data on the human diabetic immune response to any of the peptides they name, it is hard to see how any peptide could be considered as (a) relevant to human disease and (b) relevant to human therapy.

Additional scientific reasons why the Congia et al paper is flawed and in no way deprives the present invention of patentability are the following :

1. The subject is a "cloned" mouse transgenic for human HLA-DR4. It is not clear that mice generate the same peptide epitopes after cellular processing as man. Since the mice under study are products of in-breeding (i.e. brother-sister mating), the responses analyzed are essentially a "case report" and bear no relation to an outbred population.

2. The preproinsulin (PPI) used to immunize the mice is generated from SDS-PAGE gels. The PPI in this form would be denatured. Cellular antigen processing of denatured PPI may differ from that of PPI in its native, folded state.

3. Only a single cell line (c228) is used to demonstrate reactivity in Figure 3. There is no way of knowing from the data presented that this is a truly representative cell line or a one-off.

4. The PPI was administered to the mice using a powerful adjuvant. There was no attempt to analyze "natural" or "diabetes-related" responses.

5. The animal tested does not have diabetes.

In contrast to the Congia et al. disclosure, the significant epitopes disclosed in the present application have been generated in humans, shown to be dominant in the pathogenic autoimmune patients in patients with disease, and nevertheless capable of inducing a protective regulatory response of therapeutic value. Of these the preferred peptide is GSLQPLALEGSLQKRGIV. This is never tested or named in the Congia paper.

The inventive strategy of the present invention

Applicant's strategy for identifying therapeutically active peptides has made use of the novel series of selection steps described in the subject application. For the Examiner's convenience these are illustrated in outline in the attached Figure. These are:-

1. Preproinsulin peptides are eluted from the HLA-DR4 complex and those which are unique to preproinsulin are selected. (Table I of the application);

2. The selected peptides are screened for the secretion of pathogenic T cells e.g. those making IFN g (Table 3 of the application) and selected on this basis;

3. A final selection is made based on the basis of secretion of regulatory T cells e.g. those making IL-10, (Table 4 of the application).

This strategy has enabled the determination of the sequences of crucial peptides and peptide combinations effective for the therapeutic or prophylactic control of T1DM. It is apparent that an essential component sequence of these peptides is the sequence:-

QPLALEGSLQK (SEQ ID NO: 9).

which sequence is extended at one or both ends thereof in the other peptides disclosed in the application.

Although the peptides for use according to the present invention are components of the preproinsulin molecule, no therapeutic activity has previously been ascribed to these novel peptides separated from sequences with which they are associated in preproinsulin. Similarly, no therapeutic activity has been hitherto discovered for peptides having other subsequences of the preproinsulin molecule for which immunogenic properties have been disclosed, including sequences described by Congia et al. based solely on experiments in mice. In particular, it should be noted that there has been no previous agreement among those skilled in the art as to what peptide components of preproinsulin are immunodominant peptides in the human disease. In these circumstances, there has been no clear prior indication or suggestion of therapeutic activity associated with peptides having such previously disclosed subsequences.

In contrast, the peptides of the present invention have been demonstrated as having therapeutic or preventive activity especially in relation to T1DM. These peptides are especially useful for the treatment of patients that have the HLA-DR4 allele.

For use in the therapeutic or prophylactic treatment of T1DM, at least one of the above peptides will be the primary component of the pharmaceutical composition supplied for such use. Various combinations of two or more of these peptides may be used if desired. As indicated in the application as filed, one or more of the above sequences when used in combination with one or more peptides from IA-2 have now been found to exhibit good synergy with preproinsulin peptide SEQ ID NO 5.

Withdrawal of the anticipation rejections is respectfully requested.

VII. THE OBVIOUSNESS REJECTION

Claims 10 and 16 stand rejected under 35 USC 103(a) as allegedly unpatentable over Meierhoff et al. That rejection is respectfully traversed.

Meierhoff et al. reviews the value of the cytokine ELISPOT assay in relation to immunological studies on T1DM. The primary interest of these authors is the use of this method "to monitor the immune process and disease activity". Although reference is made to "visualising, monitoring, and predicting immune responses and treatment effects",

There is no disclosure of the use of ELISPOT in relation to the identification of peptides of potential value for therapy, and there is no hint or suggestion in this paper of the 3-part strategy which has been devised by the present inventor and of which the ELISPOT assay is just one part. There is no mention in the Meierhoff paper of using an ELISPOT to identify epitopes that may be relevant to diabetes development or therapy by using an approach in which the numerical balance of IFN-γ and IL-10 responder cells is measured as a gauge of tolerance. There is no mention of the use of the ELISPOT for detection of regulatory T cells that might indicate therapeutic peptides.

In the Meierhoff article, the characterization of T cells is said to be "of primary interest both for understanding the underlying pathogenic mechanism and for assessing the efficacy of therapeutic intervention." but no such intervention is described in this paper, and it is not stated that the technology might be used for the development of therapy.

The Examiner has focused attention on the mention of the IA-2 peptides disclosed in Peakman et al. and concludes that it would have been obvious to apply the ELISPOT analysis to such peptides. This leap of logic is unjustified. The IA-2 peptides *per se* are not the subject of the present application, in which they figure solely in combination with the novel PPI peptides disclosed therein. Moreover the therapeutic potential of the IA-2 peptides per se has been demonstrated previously and therefore does not require the use of the strategy of the present invention.

VIII. FINAL REMARKS

In summary, the present application provides strong support for the pursuit of a novel strategy which has enabled peptides to be identified which act as the targets of a regulatory T cell subset. These Tr1 cells have been found in patients with slowly progressive disease, thus providing *in vivo* evidence that Tr1 cells recognizing the claimed preproinsulin peptides, especially when combined with IA-2 peptides, have regulatory and tolerogenic properties. Since Tr1 cells recognizing these peptides may be induced by peptide immunotherapy, the present invention comprises the therapeutic use of these preproinsulin and IA-2 peptides in diabetes prevention.

Our strategy for peptide immunotherapy is the use of peptide injection, or related methods, to induce in vivo regulatory populations of T cells (Tr1 cells) that recognize the same peptide as the equivalent effector pathogenic T cells (these cells make IFN-γ and are called Th1). Peptide immunotherapy is particularly potent at inducing Tr1 cells that synthesize the immunosuppressive chemical mediator IL-10. Under these circumstances, when one of the preproinsulin and IA-2 peptides is presented by an

antigen-presenting cell in the pancreas or local lymph nodes in a patient developing diabetes, the peptide is recognized simultaneously by Th1 and Tr1 cells. Under such circumstances, the Tr1 cell is dominant and exercises "bystander suppression" over the Th1 response.

The identification of a specific combination of peptides that identifies pathogenic CD4+ T lymphocytes in a majority of patients leads us to a therapy by which CD4+ T lymphocytes involved in T1DM are inactivated, restoring long-term beta cell tolerance. An important element in this approach is the demonstration that the combination of 4 epitopes from 2 autoantigens is outstanding in terms of its coverage of pathogenic CD4+ T lymphocyte responses. Such coverage gives a greater potency to the therapy, and applicability to a wider range of patients, than any mono-therapy hitherto proposed. Thus our therapeutic approach is multi-epitope, multi-antigen peptide immunotherapy and uses the peptides we have identified through a combination of elution and bioassay.

The present inventor is the first to show:

(a) the presence of naturally arising regulators specific for self-peptides in any human disease, and

(b) that these cells are associated with slower onset of diabetes i.e. that they really regulate *in vivo*.

These findings have led to the use of peptides to induce the regulatory cells as a novel immunotherapy approach to the control and prevention of T1DM. It is believed that this is an invention worthy of protection as defined in the amended claims.

Favorable action on this application is awaited.

Respectfully submitted,

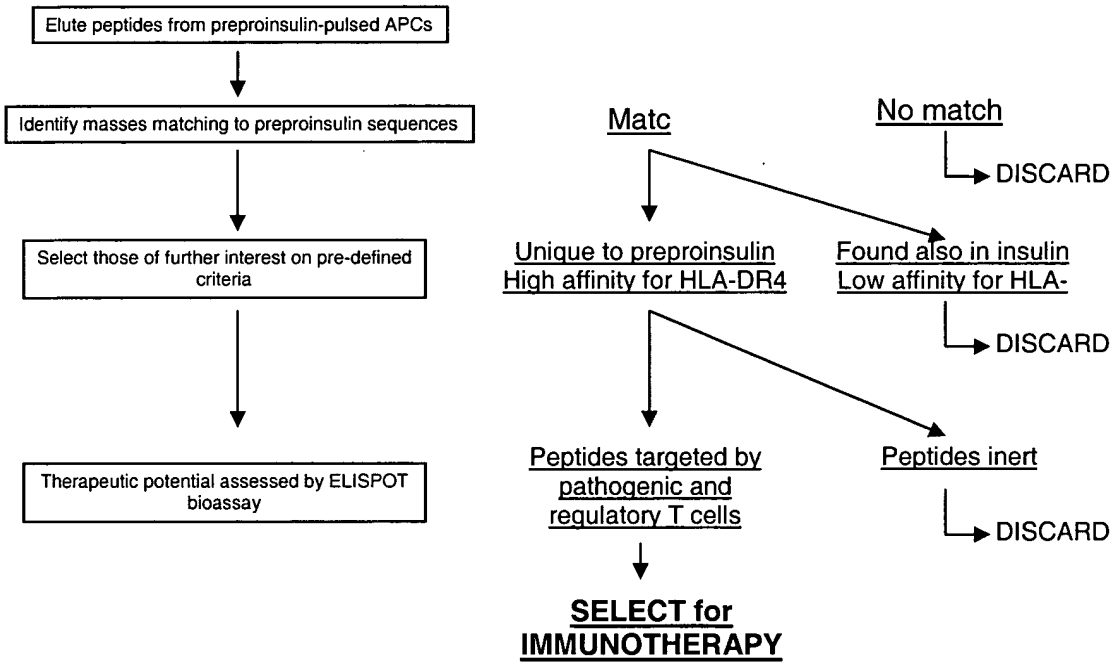
NIXON & VANDERHYE P.C.

By: _____
Leonard C. Mitchard
Reg. No. 29,009

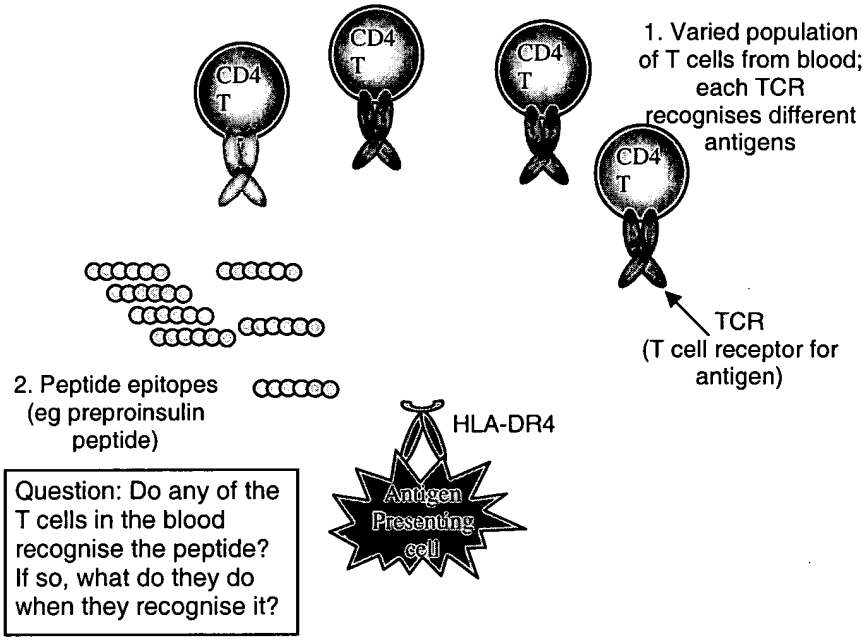
LCM:lfm
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

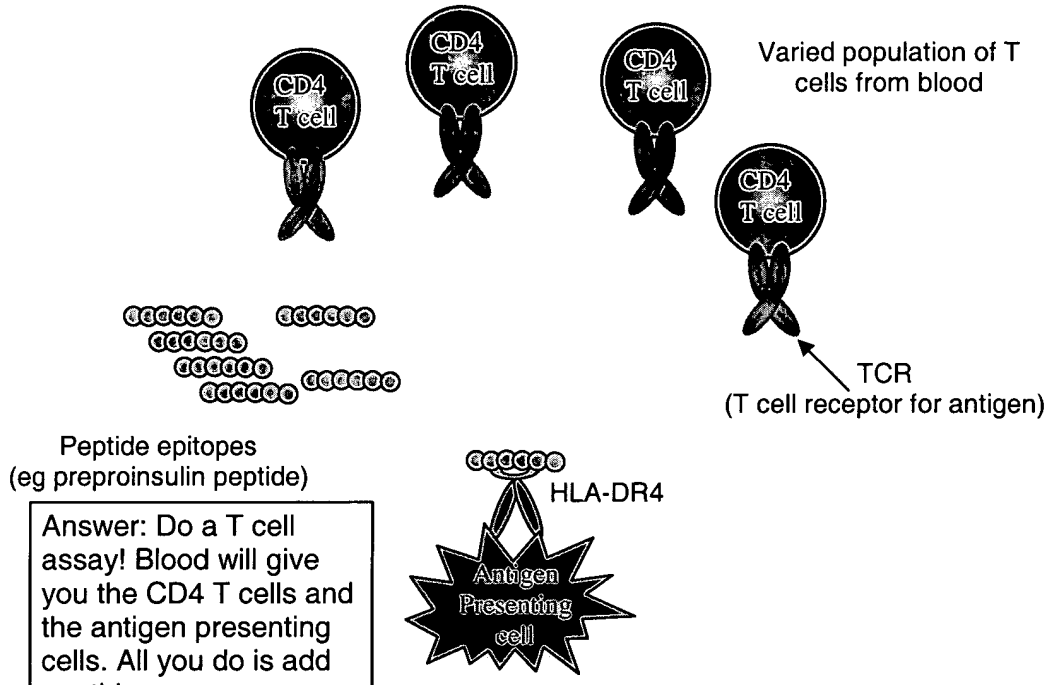
Attachment: Figure of the structure and complete amino-acid sequence thereof; IDS, PTO 1449, references; IDS fee.

Strategy for invention of preproinsulin peptide

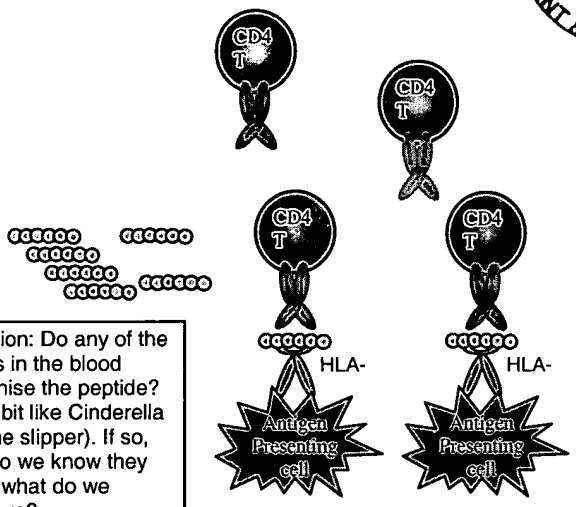


THE SCHEME BELOW IS INTENDED AS AN AIDE TO UNDERSTANDING AND NOT FOR INCLUSION IN THE OFFICIAL RESPONSE



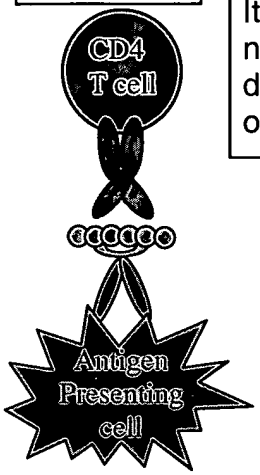


Answer: Do a T cell assay! Blood will give you the CD4 T cells and the antigen presenting cells. All you do is add peptide.



Question: Do any of the T cells in the blood recognise the peptide? (It's a bit like Cinderella and the slipper). If so, how do we know they do; ie what do we measure?

This cell responds



It now does one or a number of things, depending on what type of cell it is.

Proliferates and makes lots of daughter cells

Makes cytokines

Crucial question – what type of cytokine does it make when it responds?

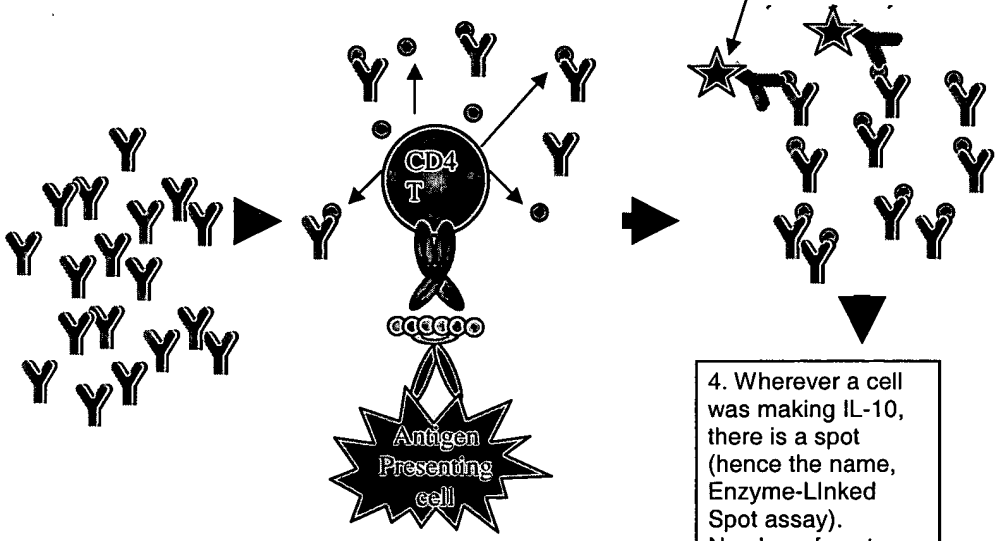
- Interferon – pro-inflammatory

How to do an

1. Coat the cell culture dish with anti-IL-10 antibody

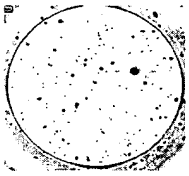
2. Cell makes IL-10 ( ) in response to peptide – it is captured by anti-IL-

3. Wash cells away and add another anti-IL-10 antibody linked to an enzyme - this catalyses



4. Wherever a cell was making IL-10, there is a spot (hence the name, Enzyme-LInked Spot assay). Number of spots =

Typical appearance of an ELISPOT assay



The key point is that you get 2 answers for one assay – does it respond, and what is the type of response (aggressive, regulatory). Thus the phrasing of the question when you interrogate T cells is critical – are you a regulator? Are you something else etc. But you can only ask one question at a time.
The patent people can argue the ELISPOT is an obvious choice of assay, but they can really only say this because it is very sensitive – it can count 1 responder cell in 1 million non-responders – and, yes, we are looking for rare cells.
In my view they cannot argue that it was obvious to look for IL-10 producing cells and relate this to diabetes onset. To my mind this was an inventive step. In our 2004 paper we were the first people ever to show (a) the presence of these naturally arising regulators specific for self-peptides in any human disease, and (b) that these cells are associated with slower onset of diabetes (ie they really regulate in vivo). Now, the peptide immunotherapy for which we want to use the peptides is designed to induce these cells. This has a logic flow to it.